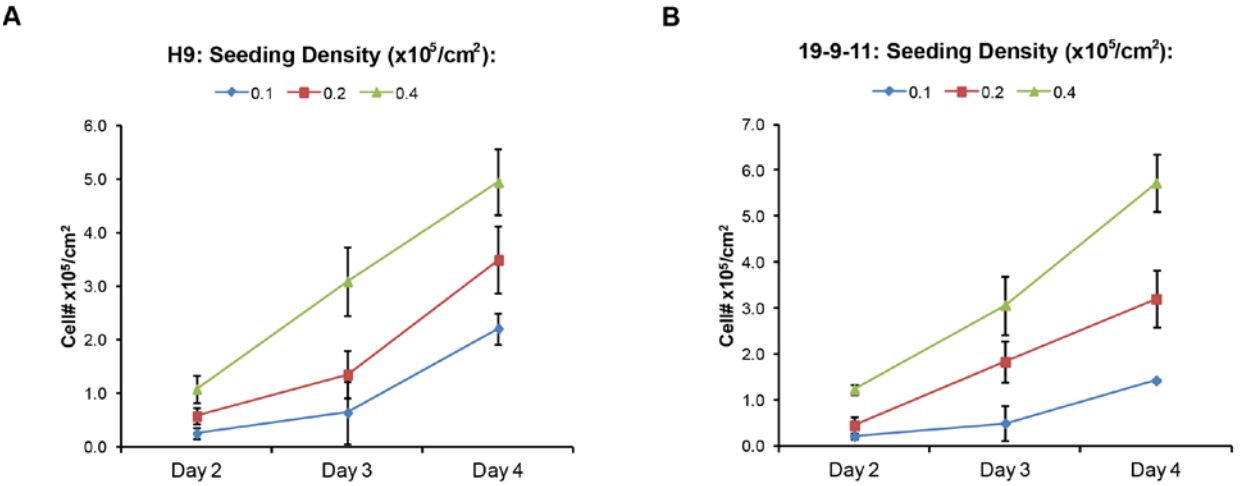
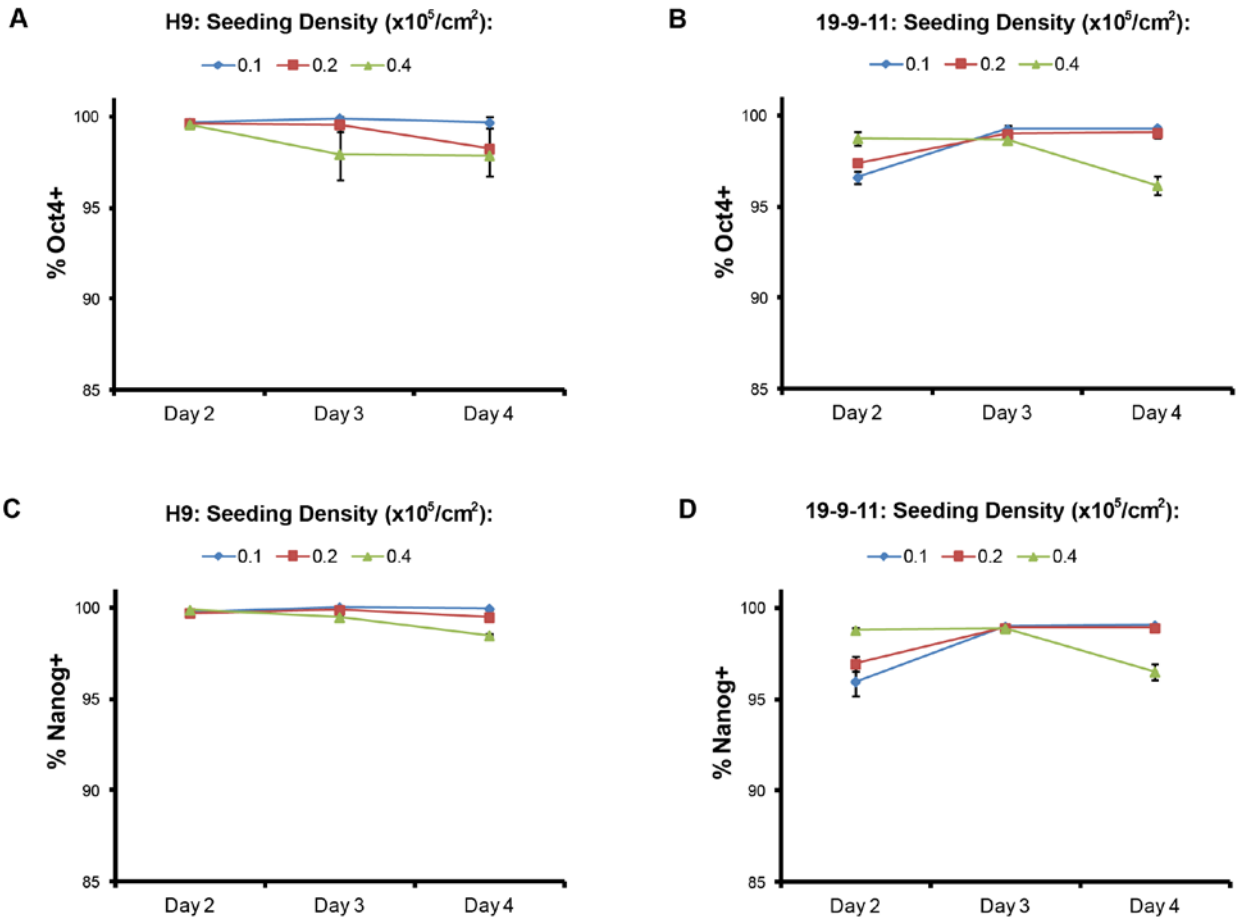
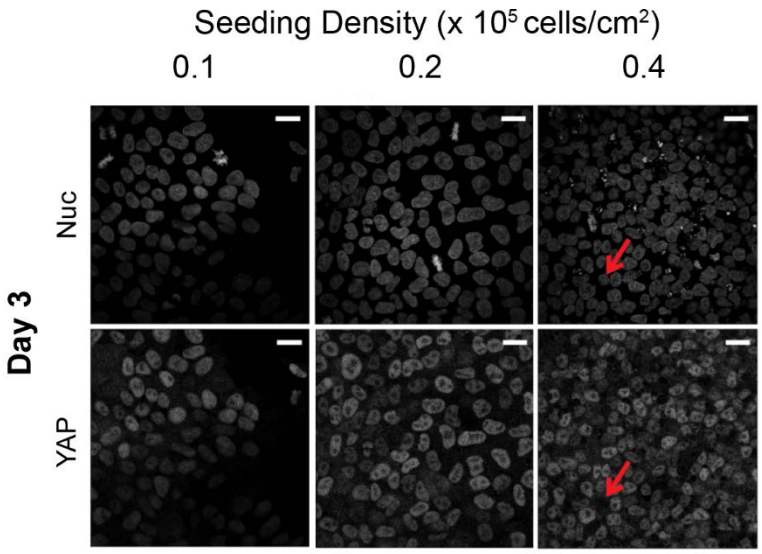
Supporting Information



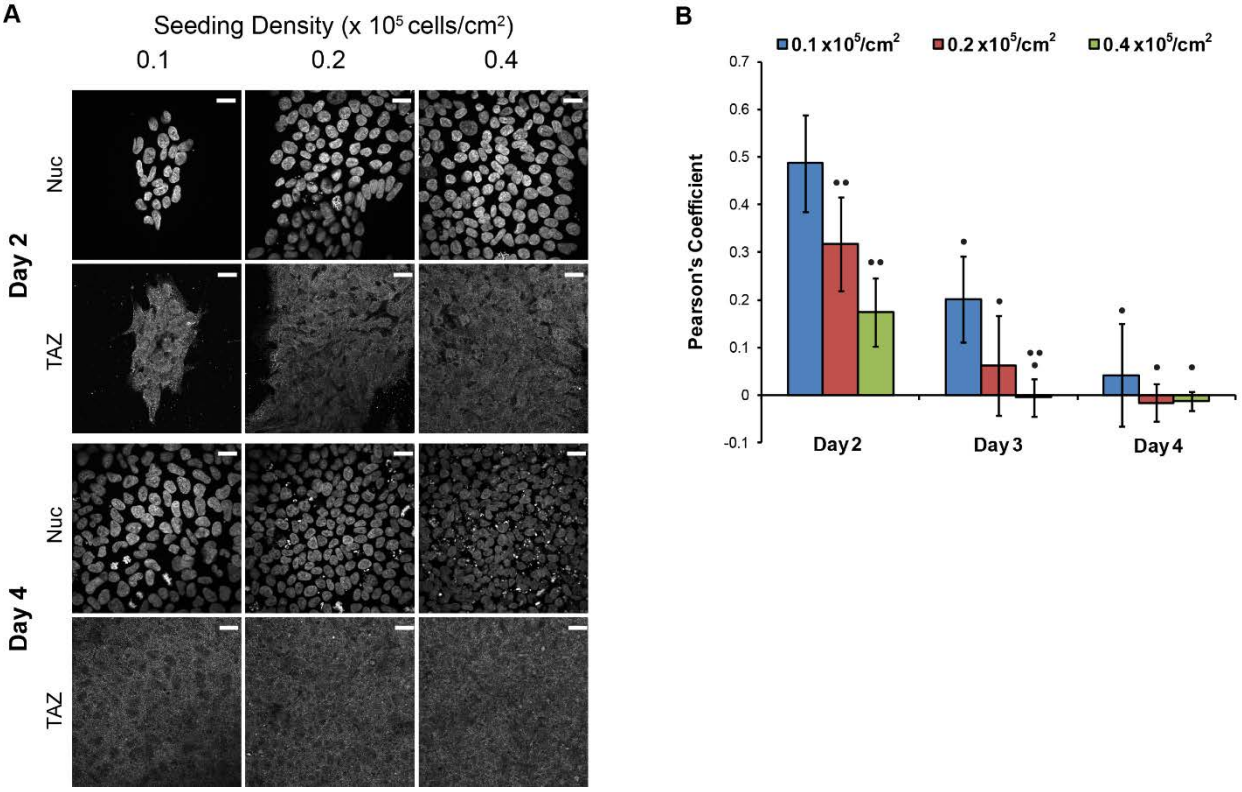
Supplemental Figure 1. Cell density increased over time across seeding densities. (A) H9 and (B) 19-9-11 cells were singularized and plated at 0.1, 0.2 and 0.4 x 10^5 cell/cm^2. After 2, 3 and 4 days of culture, cells were singularized for counting and determination of cell density. Error bars represent standard deviation of at least three independent experiments.



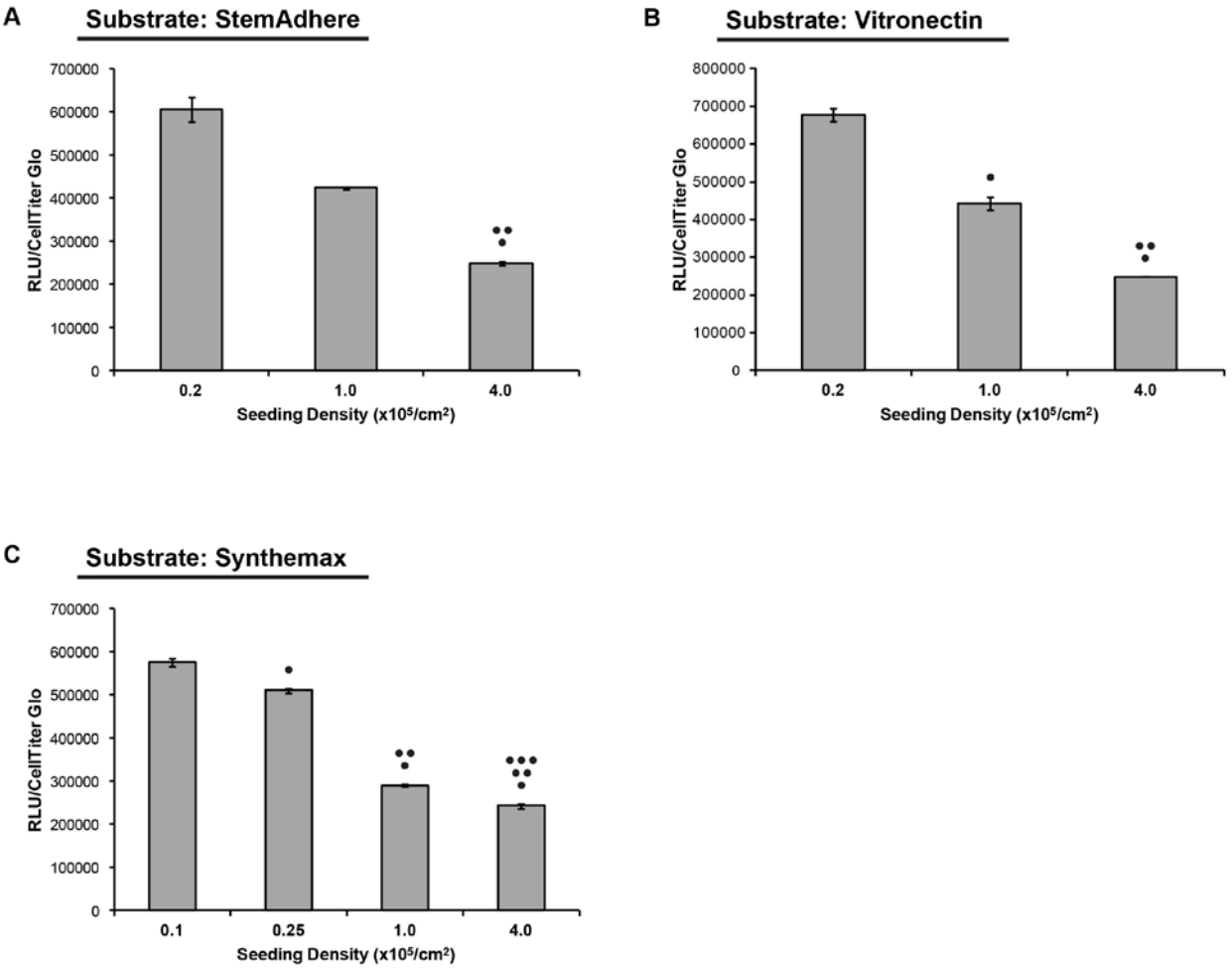
Supplemental Figure 2. hPSCs maintained expression of pluripotency markers over time across seeding densities. hPSCs were singularized and plated at 0.1, 0.2 and 0.4 x 10^5 cell/cm^2. Cells were harvested for flow cytometry after 2, 3 and 4 days of culture. Analysis of (A) % Oct+ H9 cells, (B) % Nanog H9 cells, (C) % Oct4+ 19-9-11 cells, and (D) % Nanog+ 19-9-11 cells was performed. Error bars represent standard deviation of at least three independent experiments.



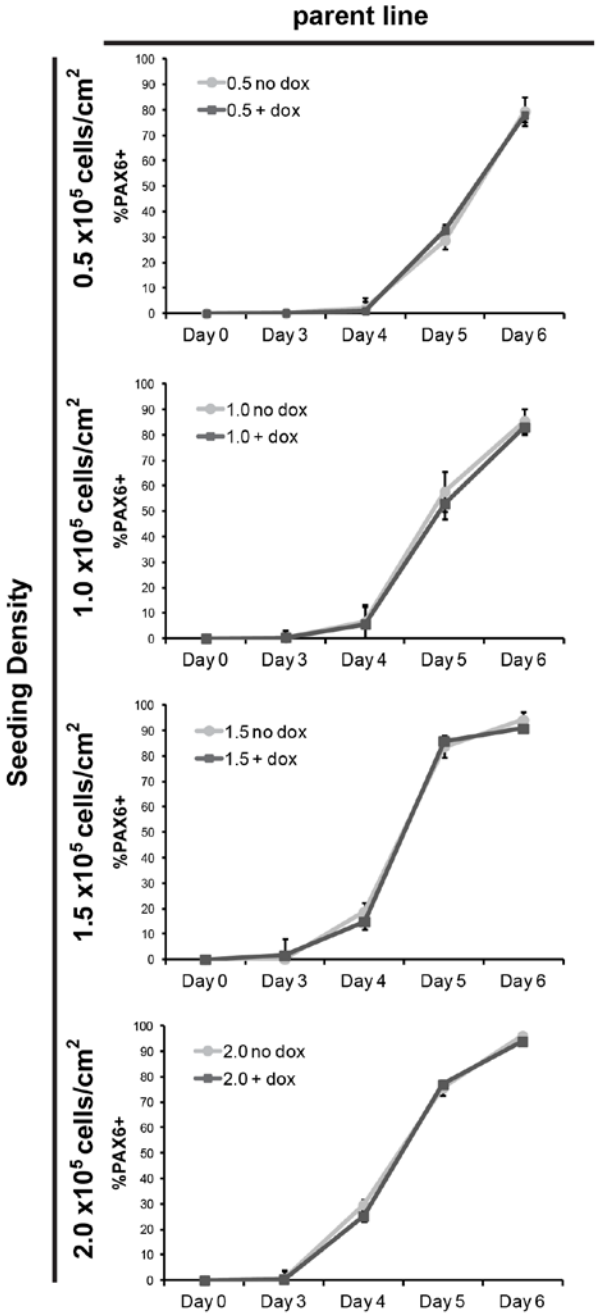
Supplemental Figure 3. YAP localization switched from nuclear to cytoplasmic as cell density increased. hPSCs were singularized and plated at 0.1, 0.2 and 0.4 x 10^5 cell/cm^2. Representative confocal images of the nuclear Hoechst stain and YAP immunofluorescence after 3 days of culture are shown. A nucleus lacking detectable YAP is pointed out by the red arrow. Scale bars = 10 µm.



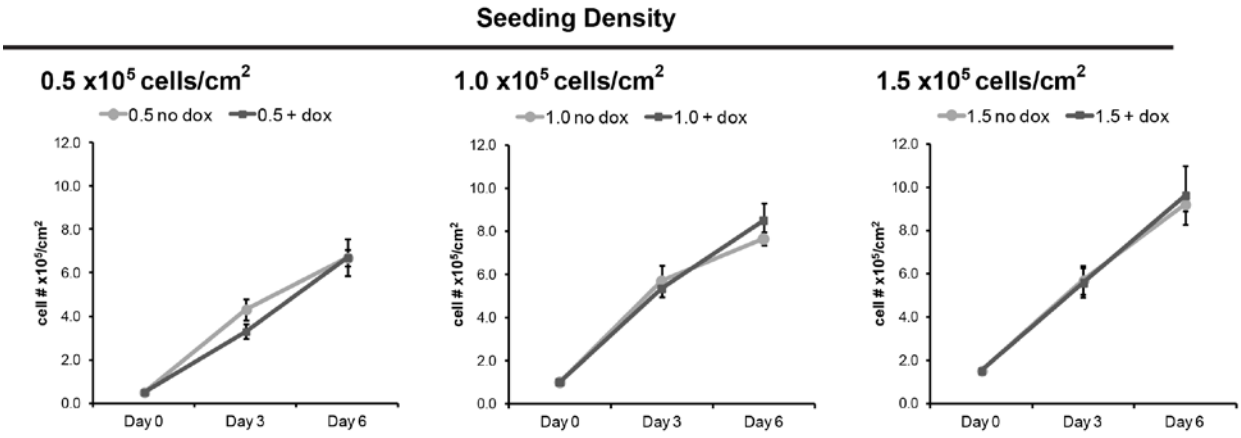
Supplemental Figure 4. TAZ localization switched from nuclear to cytoplasmic as cell density increased. hPSCs were singularized and plated at 0.1, 0.2 and 0.4 x 10^5 cell/cm^2. Cells were fixed and stained after 2, 3 and 4 days of culture. (A) Representative confocal images of the nuclear Hoechst stain and TAZ immunofluorescence at days 2 and 4 are shown. Scale bars represent 10 µm. (B) Pearson's coefficients were calculated for 5 to 7 images and averaged for each condition to quantify the colocalization of TAZ with the Hoechst stain. Pearson's coefficient of 1 represents complete colocalization, 0 represents no correlation and -1 represents negative correlation. Error bars represent standard deviation. (• indicates $p<0.05$ compared to Day 2, •• indicates $p<0.05$ compared to 0.1 x10^5 cell/cm^2 seeding density on the same day)



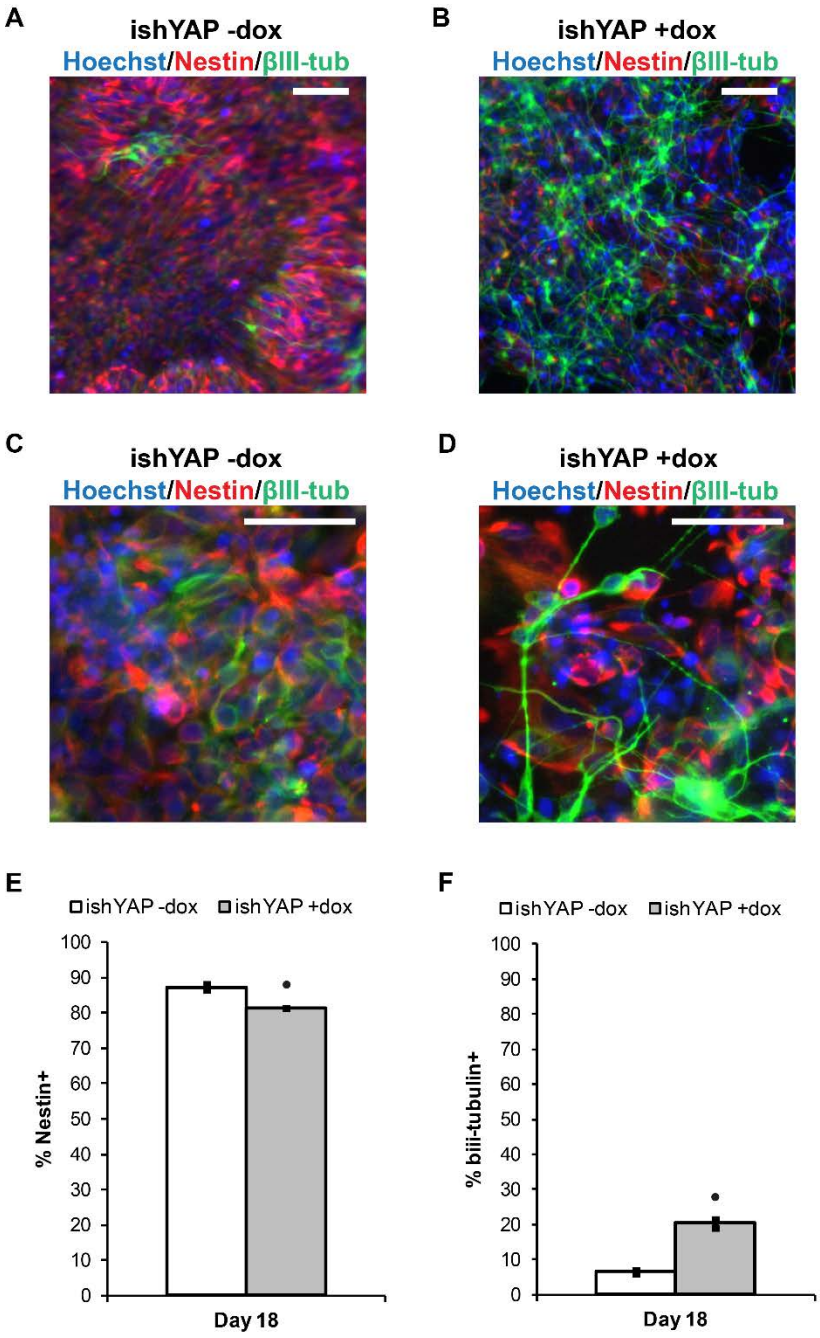
Supplemental Figure 5. YAP/TEAD transcriptional activity decreased as hPSC density increased on multiple substrates. hPSCs were singularized and plated at densities of 0.1 to 4.0 x 10^5 cell/cm^2 onto chemically defined substrates (A) StemAdhere, (B) vitronectin, and (C) Synthemax. After 3 days in culture, 50,000 cells were harvested for luciferase assays. Chemiluminescent signal was normalized to CellTiter-Glo signal. Error bars represent standard deviation of at least three independent experiments. (For substrates StemAdhere and vitronectin: • indicates $p<0.05$ compared to 0.2 x10^5 cell/cm^2 seeding density, •• indicates $p<0.05$ compared to 1.0 x10^5 cell/cm^2 seeding density. For substrate Synthemax: • indicates $p<0.05$ compared to 0.1 x10^5 cell/cm^2 seeding density, •• indicates $p<0.05$ compared to 0.25 x10^5 cell/cm^2 seeding density, ••• indicates $p<0.05$ compared to 1.0 x10^5 cell/cm^2 seeding density.)



Supplemental Figure 6. Addition of doxycycline (dox) did not alter the conversion rate of hPSCs to PAX6+ neuroepithelial cells. H9 hESCs were plated on Matrigel at densities of 0.5, 1.0, 1.5 and 4.0 x 10^5 cell/cm^2 in E8 medium and neuroepithelial differentiation was initiated the next day by changing the medium to E6. Flow cytometry for the percentage of PAX6-positive cells was performed through day 6 of differentiation. Dox was added daily to fresh medium and compared to control cells with no dox added. Error bars represent standard deviation of at least three independent experiments. (• indicates $p<0.05$ compared to no dox condition on the same-day)



Supplemental Figure 7. Addition of doxycycline (dox) did not alter the total number of cells during neuroepithelial differentiation. H9 hESC ishYAP cells were plated on Matrigel at densities of 0.5, 1.0 and 1.5 x 10^5 cell/cm^2 in E8 medium and neuroepithelial differentiation was initiated the next day by changing the medium to E6. Cell counts were performed on days 3 and 6. Dox was added daily to fresh medium and compared to control cells with no dox added. Error bars represent standard deviation of at least three independent experiments.



Supplemental Figure 8. YAP knockdown increased the conversion of hPSCs to neurons. H9 hESC ishYAP cells were plated on Matrigel at 1.0 x 10^5 cell/cm^2 in E8 medium and neuroepithelial differentiation was initiated the next day by changing the medium to E6. Dox was added daily to fresh medium and compared to control cells with no dox added. (A - D) On Day 18, immunofluorescence was performed on (A, C) cells with no dox and (B, D) cells with dox added daily. Scale bars represent 50 µm. (E, F) On Day 18, flow cytometry for percentage (E) Nestin positive cells and (F) βIII-tubulin positive cells. Error bars represent standard deviation. (• indicates $p<0.05$ compared to no dox condition)

Supplemental Table 1. List of the antibodies and their dilutions utilized for immunofluorescence (IF), western blotting (WB) and flow cytometry (FC).

Target	Company	Product #	Application
YAP	Cell Signaling Technologies	4912S	1:200 (IF) 1:1000 (WB)
phospho-YAP	Cell Signaling Technologies	4911S	1:200 (IF) 1:1000 (WB)
TAZ	BD Pharmingen	560235	1:400 (IF)
TAZ	Cell Signaling Technologies	2149S	1:1000 (WB)
Histone H3	Cell Signaling Technologies	9715	1:1000 (WB)
GAPDH	Cell Signaling Technologies	8884S	1:5000 (WB)
β-Actin	Cell Signaling Technologies	5125S	1:5000 (WB)
PAX6	Developmental Studies Hybridoma Bank (DSHB)		1µg/mL (FC)
Oct-3/4	BD Pharmingen	560186	1:200 (FC)
Nanog	BD Pharmingen	560483	1:200 (FC)

Supplemental Table 2. List of the primer sequences, annealing temperatures (T_a) and cycle numbers utilized for RT-PCR.

Gene	Primer Sequence		T_a(°C)	Cycles
TBP	fwd:	CCCGAAACGCCGAATA	57	35
	rev:	AATCAGTGCCGTGGTT		
PCTK3	fwd:	CCACAGCAACAGAAGGAATAG	57	35
	rev:	AGAGCCCTGGGATGATAAG		
SMARCA1	fwd:	GCAGTGGATGCCTACTTTAG	57	35
	rev:	GAGGTTCAGCTCCATCAATC		
SCARA3	fwd:	GGAGTGCTACGATGTCAAG	57	35
	rev:	CAGGAAGGACGAGATGTTATC		
RRM2	fwd:	CAGAGTAGAGAACCCATTTGAC	57	35
	rev:	TCACTCCCATCCTCTGATAC		
RHOF	fwd:	GCTGAAGATCGTGATC	57	35
	rev:	CTTCTCGAACACCGAT		
STXBP6	fwd:	GTGCTGTCCTCACTTGTTCTAC	57	35
	rev:	CTTTGTCCTCCTCTTGACTTGG		
YAP1	fwd:	GAACTCGGCTTCAGGTCCTC	57	35
	rev:	GGGGTGGTGGCTGTTTCACT		
TAZ	fwd:	CCTGAAGTTGATGCGTTGGACC	57	35
	rev:	TCCCCTCATTCTCTGCTTGGA		
NANOG	fwd:	CGAAGAATAGCAATGG	57	35
	rev:	TTCCAAAGCAGCCTCC		
SOX2	fwd:	CAAGATGCACAACTCG	57	35
	rev:	GTTCATGTGCGCGTAA		
POU5F1	fwd:	GAGAACAATGAGAACCTTCAGGAGA	56	30
	rev:	TTCTGGCGCCGGTTACAGAACCA		

Supplemental Table 3. Taqman probe sets for qRT-PCR.

Antibody	Brand	Assay ID
ACTB	Life Technologies	Hs01060665_g1
YAP1	Life Technologies	Hs00902712_g1